Journal of Chromatography A, 1404 (2015) 124–130

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

An analytic description of electrodynamic dispersion in free-flow zone electrophoresis

Debashis Dutta*

Department of Chemistry, (Dept. # 3838), University of Wyoming, 1000 East University Avenue, Laramie, WY 82071 USA

ARTICLE INFO

Article history:
Received 13 December 2014
Received in revised form 12 May 2015
Accepted 17 May 2015
Available online 1 June 2015

Keywords:
Band broadening
Electrodynamic dispersion
Electroosmotic cross-flow
Free-flow zone electrophoresis
Method-of-moments

ABSTRACT

The present work analyzes the electrodynamic dispersion of sample streams in a free-flow zone electrophoresis (FFZE) chamber resulting due to partial or complete blockage of electroosmotic flow (EOF) across the channel width by the sidewalls of the conduit. This blockage of EOF has been assumed to generate a pressure-driven backflow in the transverse direction for maintaining flow balance in the system. A parallel-plate based FFZE device with the analyte stream located far away from the channel side regions has been considered to simplify the current analysis. Applying a method-of-moments formulation, an analytic expression was derived for the variance of the sample zone at steady state as a function of its position in the separation chamber under these conditions. It has been shown that the increase in stream broadening due to the electrodynamic dispersion phenomenon is additive to the contributions from molecular diffusion and sample injection, and simply modifies the coefficient for the hydrodynamic dispersion term for a fixed lateral migration distance of the sample stream. Moreover, this dispersion mechanism can dominate the overall spatial variance of analyte zones when a significant fraction of the EOF is blocked by the channel sidewalls. The analysis also shows that analyte streams do not undergo any hydrodynamic broadening due to unwanted pressure-driven cross-flows in an FFZE chamber in the absence of a transverse electric field. The noted results have been validated using Monte Carlo simulations which further demonstrate that while the sample concentration profile at the channel outlet approaches a Gaussian distribution only in FFZE chambers substantially longer than the product of the axial pressure-driven velocity and the characteristic diffusion time in the system, the spatial variance of the exiting analyte stream is well described by the Taylor–Aris dispersion limit even in analysis ducts much shorter than this length scale.

1. Introduction

Free-flow zone electrophoresis (FFZE) is an important technique useful in the isolation/purification of target analytes from relatively large liquid samples either for preparative applications or for allowing their further downstream analysis [1,2]. The continuous fractionation realizable using this approach is particularly suitable for analyzing biological mixtures due to its high throughput and reliance on relatively gentle operating conditions [3–5]. In an FFZE device, a pressure-drive is commonly used to introduce a co-currently flowing sample and buffer stream through a region with a transverse electric field [6–12]. The electric field causes the different constituents of the sample mixture to deflect from their original flow direction based on their electrophoretic mobilities. As a result, these individual analytes exit the FFZE chamber at different lateral positions leading to the desired fractionation process. As with any other electrophoretic technique, the resolving power of FFZE assays is often limited by the broadening of the analyte streams as they migrate through the analysis channel [13–19]. Such broadening primarily occurs due to molecular diffusion orthogonal to the flow direction as well as a variation in the transit time for the analyte molecules when travelling along the different streamlines in the system. While the first contribution to stream broadening is relatively simple to estimate, the latter one is determined by a complex interplay between the local streamline velocity and analyte diffusion across these streamlines [20–25]. It has been established that the latter effect is particularly prominent at moderate to large transverse electric fields [26] and can originate for a variety of reasons. The parabolic pressure-driven flow profile along the channel length, for example, leads to longer transit times for molecules traveling close to the top and bottom channel walls (parallel-plates) compared to those migrating along the channel center [26–29]. Consequently, they are electrophoretically deflected to a larger extent in the lateral direction giving rise to

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* Tel.: +1 307 766 4318; fax: +1 307 766 2807.
E-mail address: ddutta@uwyo.edu

http://dx.doi.org/10.1016/j.chroma.2015.05.035
0021-9673/

the hydrodynamic dispersion component in FFZE assays. The overall variance (σ^2) of analyte zones under these conditions may be described using the following analytic expression [29]

$$\sigma^2 = \underbrace{\frac{2DL}{U}}_{\text{diffusive broadening}} + \underbrace{\frac{2\mu^2E^2DL}{U^3}}_{\text{orientation factor}} + \underbrace{\frac{1}{105}\frac{\mu^2E^2d^2L}{UD}}_{\text{hydrodynamic broadening}} + \underbrace{\frac{b^2}{12}}_{\text{injection variance}} \qquad (1)$$

where D, U and μ refer to the molecular diffusion coefficient, spatially averaged axial pressure-driven velocity and net electrokinetic mobility (algebraic sum of the electroosmotic and electrophoretic mobilities) of the analyte species, respectively. The symbols d and L here represent the depth and length of the FFZE compartment, E refers to the transverse electric field in the system and b correspond to the width of the sample stream at the channel entrance. Eq. (1) shows that in addition to the diffusive and hydrodynamic broadening components, there are two other contributions to σ^2 even under ideal operating conditions. The first among these is captured by the term $2\mu^2E^2DL/U^3$ (orientation factor) which arises from the fact that σ is measured perpendicular to the flow direction of the sample/buffer stream whereas the analyte zone makes an angle $\theta = \tan^{-1}\left(\mu E/U\right)$ with respect to this axis. The other contribution comes about from the finite injection width of the sample stream at the channel entrance and is quantitated by the term $b^2/12$. Interestingly, if one compares the different contributions to σ^2 for a fixed migration distance of the analyte zone in the lateral direction ($S = \mu EL/U$), Eq. (1) translates to

$$\begin{aligned}\sigma^2 &= \frac{2DL}{U} + \frac{2S^2D}{UL} + \frac{1}{105}\frac{S^2d^2U}{DL} + \frac{b^2}{12}\\ &= \underbrace{\frac{b^2}{12}}_{A} + \underbrace{\left(2DL + \frac{2S^2D}{L}\right)\frac{1}{U}}_{B} + \underbrace{\frac{1}{105}\frac{S^2d^2}{DL}U}_{C} \qquad (2)\end{aligned}$$

yielding an expression similar to the van Deemter equation [30] commonly used for describing sample dispersion in band separations. Recent experimental works [11,26] have further shown that the C term in Eq. (2) tends to dominate the overall variance of analyte zones at moderate to large electric fields when EOF occurs in the direction of sample electrophoresis. Moreover, these experiments also validate the quadratic dependence of this term on the lateral migration distance of the sample stream (S) [26,29] building further confidence into these results. Unfortunately however, the coefficient multiplying the ratio S^2d^2/DL in the C term was estimated to be about 15 times larger than the theoretical value 1/105 in the reported FFZE assays [29]. In this situation, a quantitative understanding of other possible sources of stream dispersion becomes necessary in order to establish the design rules for the FFZE technique. Previous scientific literature indicates that stream broadening in FFZE fractionations can also occur due to several non-idealities in the system. These include Joule heating effects [31], difference in the electrical conductivity between the sample and buffer streams (electrohydrodynamic dispersion) [32–34] and/or pressure-driven cross-flow arising from complete/partial blockage of EOF by the channel side-walls (electrodynamic dispersion) [35], among others. The focus of this article is to develop a quantitative understanding of the electrodynamic dispersion component based on the transport equations applicable to FFZE systems. A method-of-moments formulation [29,36] has been used to theoretically study the effect of this phenomenon on the variance of the sample stream as a function of the different operating parameters.

2. Mathematical formulation

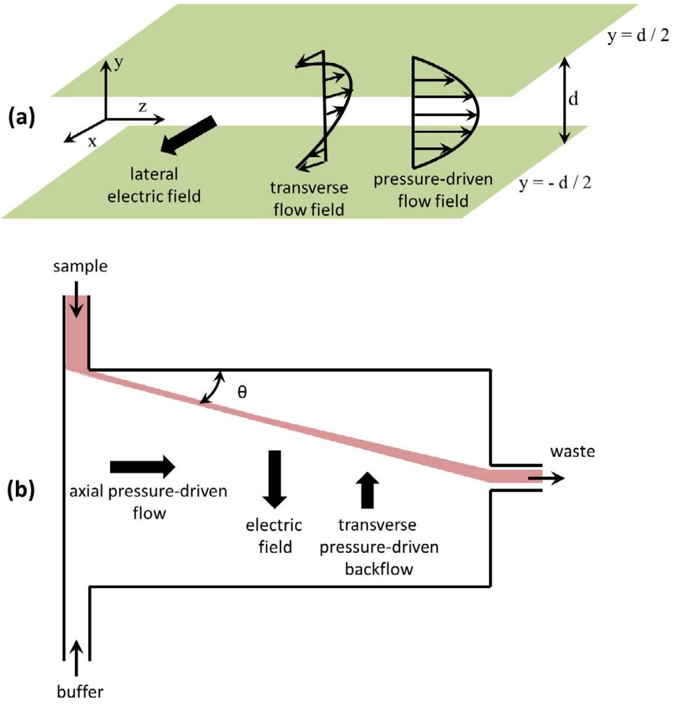

Fig. 1. (a) Schematic of the FFZE fractionation process with a pressure-driven backflow in the transverse direction as described in this article. (b) Top view of a microfluidic FFZE device relevant to the mathematical analysis presented in this work.

To evaluate electrodynamic dispersion in an FFZE system, the flow of an analyte stream between two parallel plates separated by a distance d (see Fig. 1) has been considered. This flow occurs due to the combined effects of an applied pressure-gradient in the axial direction (along the z-coordinate) and an external electric field (E) across the width of the separation chamber (along the x-coordinate). In order to simplify the mathematical analysis, the location of the parallel plates in the present work are set to $y = \pm d/2$ yielding a pressure-driven velocity profile $u_p = \left(3U/2\right)\left(1 - 4y^2/d^2\right)$ with U being the spatially averaged value of u_p. In addition, a fraction (α) of the net transverse electrokinetic flow is assumed to be blocked by the channel sidewalls, which then yields as a pressure-driven cross-flow countering the EOF in the FFZE channel [28]. The overall flow profile in the transverse direction in this situation may be expressed as $u_t = \mu E\left[1 - \left(3\alpha/2\right)\left(1 - 4y^2/d^2\right)\right]$ in regions far away from the channel sidewalls. Notice that the spatially averaged value of u_t in this description equals $\mu E(1-\alpha)$ which determines the lateral migration distance for the analyte stream at the channel exit. The transport equation governing the concentration of sample species (C) at steady state under these conditions may be written as

$$\begin{aligned}&\mu E\left[1 - \frac{3}{2}\alpha\left(1 - \frac{4y^2}{d^2}\right)\right]\frac{\partial C}{\partial x} + \frac{3U}{2}\left(1 - \frac{4y^2}{d^2}\right)\frac{\partial C}{\partial z}\\ &= D\left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2}\right) \qquad (3)\end{aligned}$$

Upon normalizing all length scales with respect to d, i.e., x^*, y^*, $z^* = x/d, y/d, z/d$, and the sample concentration by its inlet value (C_0), i.e., $C^* = C/C_0$, Eq. (3) may be reduced to the dimensionless form

$$\begin{aligned}&\frac{Pe_x}{(1-\alpha)}\left[1 - \frac{3}{2}\alpha\left(1 - 4y^{*2}\right)\right]\frac{\partial C^*}{\partial x^*} + \frac{3}{2}Pe_z\left(1 - 4y^{*2}\right)\frac{\partial C^*}{\partial z^*}\\ &= \frac{\partial^2 C^*}{\partial x^{*2}} + \frac{\partial^2 C^*}{\partial y^{*2}} + \frac{\partial^2 C^*}{\partial z^{*2}} \qquad (4)\end{aligned}$$